U.S. Application No. 10/722,371
Inventors: Qin et al.

Title: Methods of Producing and Purifying Recombinant Alpha-LIduronidase
Docket No. 30610/30009A
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| 10                | 20                | 30                | 40<br>* *         | 50<br>* *         | 60<br>* *          | 70<br>* *         |
|-------------------|-------------------|-------------------|-------------------|-------------------|--------------------|-------------------|
|                   |                   |                   |                   |                   | TGCTCTGATG         | CCGCATAGTT        |
| 80<br>* *         | 90<br>* *         | 100<br>* *        | 110<br>* *        | 120<br>* *        | 130<br>* *         | 140<br>* *        |
| AAGCCAGTAT        | ствстссств        | CTTGTGTGTT        |                   |                   | CGAGCAAAAT         |                   |
| 150<br>* *        | 160<br>* *        | 170<br>* *        | 180               | 190<br>* *        | 200<br>* *         | 210<br>* *        |
|                   |                   |                   |                   |                   | GCGTTTTGCG         |                   |
| 220<br>* *        | 230<br>* *        | 240<br>* *        | 250<br>* *        | 260<br>* *        | 270<br>* *         | 280<br>* *        |
|                   |                   |                   |                   |                   | TAGTAATCAA         |                   |
| 290<br>* *        | 300<br>* *        | 310<br>* *        | 320<br>* *        | 330<br>* *        | 340<br>* *         | 350<br>* *        |
|                   | AGCCCATATA        |                   |                   |                   |                    | TGGCTGACCG        |
| 360<br>* *        | . 370<br>* *      | 380<br>* *        | 390<br>* *        | 400<br>* *        | 410<br>* *         | 420<br>* *        |
|                   |                   |                   |                   |                   | AACGCCAATA         |                   |
| 430<br>* *        | 440<br>* *        | 450<br>* *        | 460<br>* *        | 470<br>* *        | 480<br>* *         | 490<br>* *        |
|                   |                   |                   |                   |                   | CATCAAGTGT         |                   |
| 500<br>* *        | 510<br>* *        | 520<br>* *        | 530<br>* *        | 540<br>* *        | 550<br>* *         | 560<br>* *        |
| AAGTACGCCC<br>570 | CCTATTGACG<br>580 | TCAATGACGG<br>590 | TAAATGGCCC 600    | GCCTGGCATT<br>610 | ATGCCCAGTA 620     | CATGACCTTA<br>630 |
| * *               | * *               | * *               | * *               | * *               | * *                | * *               |
| TGGGACTTTC 640    | CTACTIGGCA 650    | GTACATCTAC 660    | GTATTAGTCA<br>670 | TCGCTATTAC 680    | CATGGTGATG<br>690  | CGGTTTTGGC 700    |
| * *               | * *               | * *               | * *               | * *               | * *                | * *               |
| AGTACATCAA 710    | TGGGCGTGGA<br>720 | TAGCGGTTTG 730    | ACTCACGGGG<br>740 | ATTTCCAAGT 750    | CTCCACCCCA 760     | TTGACGTCAA<br>770 |
| * *               | * *               | * *               | * *               | * *               | * *                | * *               |
| TGGGAGTTTG<br>780 | TTTTGGCACC 790    | AAAATCAACG<br>800 | GGACTTTCCA<br>810 | AAATGTCGTA<br>820 | ACAACTCCGC<br>830  | CCCATTGACG<br>840 |
| * *               | * *               | * *               | * *               | * *               | * *                | * *               |
| CAAATGGGCG<br>850 | GTAGGCGTGT<br>860 | ACGGTGGGAG<br>870 | GTCTATATAA<br>880 | GCAGAGCICT<br>890 | CTGGCTAACT 900     | AGAGAACCCA<br>910 |
| * *               | * *               | * *               | * *               | * *               | * *                | * *               |
| 920               | GGCTTATCGA<br>930 |                   |                   |                   | AGCTTCGCAG<br>970  |                   |
| * *               | * *               | * *               | * *               | * *               | * *                | * *               |
| 990               | 1000              | 1010              |                   |                   | AGTGGCGCAT<br>1040 | 1050              |
| * *.              | * *               | * *               |                   | * *               |                    | * *               |
| 1060              |                   | 1080              |                   |                   | TCACAGGTGA<br>1110 | 1120              |
| * *               | * *               | * *               | * *               | * *               |                    | * *<br>TCCTCAACTA |
| 1130              | 1140              | 1150              | 1160              | 1170              |                    | 1190              |
| * *               | * *<br>TCCTTATATC | * *<br>ACAGAGGGAA | * *<br>ATTGGAGCTA | * *               | * * TGCCCAGAAG     | * *<br>GGAAGGGCAG |
| ACTUCIONIU        | ICCITATATC        |                   | ATTGGAGCIA        | . C I GAGGAAC     | . occononna        | Garmadacha        |

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|            | 1<br>*    | 200        |          | , 12<br>*       | 10        | *          | 122         | 0            | *           | 1230              |      | , 1<br>*  | 240<br>*        |            | 12<br>*    | 50<br>*     | ,           | 1260          |
|------------|-----------|------------|----------|-----------------|-----------|------------|-------------|--------------|-------------|-------------------|------|-----------|-----------------|------------|------------|-------------|-------------|---------------|
| AGG        |           | TTG<br>270 | стст     |                 | GT (      | CTGAG      | CCAT        |              | тстт        | CTTT<br>1300      |      |           | CCA<br>310      | GTGA       |            | CT :        | TCCCA       | 1330          |
| GGT        | *<br>CCAC | *<br>CTG   |          | *<br>CGCC       | *<br>GC ( |            | GAGG        |              | *<br>TGGC   |                   |      | *<br>GAGC | .*<br>TCT       | TGTC       | *<br>CCTG  | *<br>AC /   | *<br>ATGCC  | *<br>TGGTG    |
|            |           | 340        |          |                 | 50<br>*   | *          | 136         |              |             | 1370              |      |           | 380             | _          |            | 90          | *           | 1400          |
| CGA        |           |            | ACCC.    |                 |           | AGTGC      | TGGT        | G CG         |             |                   |      |           |                 | GGAG       |            |             | CAGA        | AAGCT         |
|            | *         | 410        |          | *               | 20 .<br>* | *          |             | *            | *           | 1440              |      | *         | 450<br>*        |            | *          | 60<br>*     | *           |               |
| ACC        |           | GTT<br>480 | TGAG     | CCCC<br>14<br>+ |           | AAGGA      | 1500        |              |             | GAGC<br>1510<br>* |      |           | TAC<br>520<br>* | CTGG       | TGAC<br>15 |             | CGTG<br>*   | TTGCG<br>1540 |
| TGT        | ATCA      |            | GAAA     |                 |           | TATCG      |             | C CG         |             |                   |      |           |                 |            |            |             |             | CAGTC         |
|            | *         | 550<br>*   |          | *               | 560<br>*  |            | *           |              | *           | *                 |      | 80<br>*   |                 | *          | 590<br>*   |             | *           |               |
| CCC        | SAGÇ      | ACG        | CGTG     |                 |           | CGT Arg    |             |              |             |                   |      |           |                 |            |            |             |             | CTG<br>Leu    |
| 1600<br>*  | *         | 1          | 610<br>* |                 | *         | 1620       |             | *            | -           | 30<br>*           | *    | 1         | 640<br>*        |            |            | 1650        | )           | *             |
| GCC        | TCG       | СТС        | CTG      | GCC             | GCG       | ccc        | CCG         | GTG          | GCC         |                   | GCC  | GAG       | GCC             | CCG        | CAC        |             |             |               |
| Ala<br>166 |           | Leu        |          | A1a<br>570      | Ala       | Pro        | Pro<br>1680 | Val          | Ala         | Pro<br>16         |      | Glu       |                 | Pro<br>700 | His        | Leu         | Val<br>1710 |               |
| 272        | *         | *          |          | *               |           | *          | *           |              | *           |                   | *    | *         |                 | *          |            | *           | *           |               |
|            |           |            |          |                 |           | CTG<br>Leu |             |              |             |                   |      |           |                 |            |            |             |             |               |
| *          | 172       |            |          | _               | 730       |            | -           | 740          |             |                   | 17   |           |                 | _          | 760        |             | ,           | 1770          |
|            | ССС       | CCG        | CTG      | CCA             |           | AGC        | CAG         |              | GAC         | CAG               | TAC  | GTC       | СТС             | AGC        |            | GAC         | . CAG       | CAG           |
| Cys        | Pro       | Pro        |          | Pro             |           | Ser<br>790 | Gln         |              | Asp<br>1800 |                   | Tyr  | Val<br>18 |                 | Ser        | •          | As p<br>820 | Gln         | Gln           |
|            | *         | 17         | *        | *               | 1         | *          |             | *            | *           |                   | *    | 10        | *               | *          | 10         | *           |             | *             |
|            |           |            |          |                 |           | GGC        |             |              |             |                   |      |           |                 |            |            |             |             |               |
| 1830       | ASII      | Leu        | 184      | -               | vai       | Gly<br>1   | 850         | vai          |             | 1860              | -    | ыу        | 11e             | -          | GIN        |             | 880         | ınr           |
| *          |           | *          |          | *               | *         |            | *           |              | *           | *                 |      | . *       |                 | *          | *          |             | *           |               |
|            |           |            |          |                 |           | GTC<br>Val |             |              |             |                   |      |           |                 |            |            |             |             |               |
|            |           |            |          |                 |           |            |             |              |             |                   | 1920 |           |                 |            |            |             |             |               |
| *          | *         | 400        | *        |                 | *         | *          | T 4 C       | *            |             | *                 | *    | •         | *               |            | *          | *           |             | *             |
|            |           |            |          |                 |           | GGG<br>Gly |             |              |             |                   |      |           |                 |            |            |             |             |               |
|            | 1         | 1950       |          |                 |           | 60         |             |              | 970         | -                 |      | 1980      |                 |            | 19         | 90          |             | -             |
| GGG        | *<br>TTT  | *<br>GAG   | CTG      | *<br>ATG        | GGC       | *<br>AGC   | ecc<br>*    | ፕ <b>Ր</b> ଜ | rer<br>*    | ĊΔC               | *    | *<br>Δ°T  | GΔC             | *<br>TTT   | GAG        | *           | *<br>۵۵6    | CAG           |
|            |           |            |          |                 |           | Ser        |             |              |             |                   |      |           |                 |            |            |             |             |               |

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| 2000                              | 2010                                    | 2020                                      | 2030                               | 2040  | 2050  |
|-----------------------------------|---|---|------------------------------------|---|---|
|                                   |   | Lys Asp Leu                               | Val Ser Ser                        |   | GA TAC ATC GGT AGG rg Tyr Ile Gly Arg 00 2110             |
| Tyr Gly                           | Leu Ala His                             |   |                                    | e Glu Thr Trp A<br>2150                           | AT GAG CCA GAC CAC<br>sn Glu Pro Asp His<br>2160<br>* *   |
| CAC GAC<br>His Asp<br>2170<br>* * | TTT GAC AAC<br>Phe Asp Asn<br>2180<br>* | GTC TCC ATG<br>Val Ser Met<br>2190<br>* * | ACC ATG CAA<br>Thr Met Glr<br>2200 | A GGC TTC CTG A<br>n Gly Phe Leu A<br>2210<br>* * | AC TAC TAC GAT GCC<br>sn Tyr Tyr Asp Ala<br>2220<br>* * * |
|                                   |   |   | Ser Pro Ala                        |   | GA GGC CCC GGC GAC  ly Gly Pro Gly Asp  0 2280  * * *     |
|                                   | His Thr Pro                             | Pro Arg Ser                               |                                    |   | TG CGC CAC TGC CAC<br>eu Arg His Cys His<br>2330 2340     |
| GAC GGT<br>Asp Gly                | ACC AAC TTC<br>Thr Asn Phe<br>2350      | TTC ACT GGG<br>Phe Thr Gly<br>2360        | GAG GCG GGG<br>Glu Ala Gly<br>2370 | C GTG CGG CTG G<br>Val Arg Leu A<br>2380          | AC TAC ATC TCC CTC<br>sp Tyr Ile Ser Leu<br>2390<br>* * * |
| CAC AGG<br>His Arg<br>2400<br>*   | AAG GGT GCG<br>Lys Gly Ala<br>2410      | CGC AGC TCC<br>Arg Ser Ser<br>2420        | ATC TCC ATC<br>Ile Ser Ile<br>2430 | e Leu Glu Gln G                                   | AG AAG GTC GTC GCG<br>lu Lys Val Val Ala<br>2450          |
| CAG CAG<br>Gln Gln<br>2460        | ATC CGG CAG<br>Ile Arg Gln<br>24        | Leu Phe Pro<br>70 2                       | Lys Phe Ala<br>480                 | Asp Thr Pro I<br>2490                             | TT TAC AAC GAC GAG<br>le Tyr Asn Asp Glu<br>2500 2510     |
| Ala Asp                           |   |   | Leu Pro Gli<br>2540                | n Pro Trp Arg A<br>2550                           | CG GAC GTG ACC TAC<br>la Asp Val Thr Tyr<br>2560          |
| GCG GCC<br>Ala Ala<br>2570        | Met Val Val<br>2580                     | Lys Val Ile<br>2590'                      | Ala Gln His<br>2600                | s Gln Asn Leu L<br>2610                           | * * * TA CTG GCC AAC ACC eu Leu Ala Asn Thr 2620          |
| Thr Ser<br>2630                   | Ala Phe Pro<br>2640                     | Tyr Ala Leu<br>26                         | Leu Ser Asi<br>50                  | n Asp Asn Ala P<br>2660 26                        | TC CTG AGC TAC CAC he Leu Ser Tyr His 70 2680             |
| *<br>CCG CAC<br>Pro His           | * * CCC TTC GCG Pro Phe Ala             | CAG CGC ACG                               | * * CTC ACC GCC Leu Thr Al         | * * G CGC TTC CAG G a Arg Phe Gln V               | * * * * TC AAC AAC ACC CGC al Asn Asn Thr Arg             |

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|     | *               | 2           | 690 ·      |            | *          | 2700<br>*  |            | *          | 27         | 10         | *          | 2          | 720<br>*   |            | *          | 2730<br>*              |     | *          |            |
|-----|-----------------|-------------|------------|------------|------------|------------|------------|------------|------------|------------|------------|------------|------------|------------|------------|------------------------|-----|------------|------------|
|     | Pro             | Pro         | His        |            |            | Leu        |            | Arg        | Lys        |            | Va1<br>70  |            | Thr        |            |            | GGG<br>Gly             |     |            |            |
|     |                 | Leu         |            | Glu        |            |            | Leu        |            |            |            |            | Ser        |            | Ala.       |            | ACC<br>Thr             | Val |            |            |
|     |                 | AAC         | CAC<br>His |            | ۷al        |            |            | Leu        |            |            |            |            | Arg        |            | Gln        | GGC<br>Gly<br>900      | CCG | GCC<br>Ala |            |
|     |                 |             |            | Ala        |            | Val        |            |            | Tyr        |            |            |            |            | Thr        |            | GCC<br>Ala<br>29       |     |            |            |
| 1   |                 |             |            | GCG        | GTG<br>Val |            | CTG<br>Leu |            | ĊTG        | CGC<br>Arg |            | GTG        |            |            | Gly        | CCG<br>Pro             | Gly |            |            |
| 7   | TAC<br>Tyr      | Va1<br>3030 | ACG        |            | TAC        | CTG<br>Leu | Asp        | AAC<br>Asn |            | СТС        | TGC<br>Cys |            | ССС        |            | GGC        | GAG<br>G1u<br>70       |     | CGG<br>Arg | Arg<br>080 |
| . ( |                 | Gly<br>3    |            |            |            |            | CCC<br>Pro |            | Ala        |            |            | TTC<br>Phe |            |            |            | *<br>CGC<br>Arg<br>313 | Ala |            |            |
|     | Asp             |             | Val        |            |            |            |            | Arg        |            | Leu        |            |            | Gly        |            |            | CTG<br>Leu             |     | Leu        |            |
|     | CCC<br>Pro      |             | CTG        | CGG<br>Arg |            | CCG        |            | CTT        | TTG<br>Leu |            | GTG<br>Val |            | GTG        | TGT<br>Cys |            | CGC<br>Arg             |     | GAG        | AAG<br>Lys |
|     |                 | Pro         |            | CAG        | Val        |            | CGG        |            | CGC        | GCC<br>Ala |            | Pro        |            | ACC        | CAA<br>Gln | GGG<br>Gly<br>3300     |     |            | GTT        |
|     | CTG<br>.eu<br>) |             | Trp        |            | GAT        | GAA<br>Glu |            | GTG        |            | TCC        | AAG<br>Lys |            | CTG<br>Leu |            | ACA        | TAC<br>Tyr             |     | ATC        |            |
| 7   | ТС              | TCT         |            | GAC        |            | AAG        | GCG        |            | ACC        |            | GTC        | AGC        |            | AAG        |            | TCG<br>Ser             | ACC |            | AAC        |

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| 3370                              | 3380                              | 3390  | 341                               | 00                                | 3410                       | 3420               |
|-----------------------------------|-----------------------------------|---|-----------------------------------|-----------------------------------|----------------------------|--------------------|
| Leu Phe Va<br>3430                | l Phe Ser P<br>344                |   | Gly Ala Val<br>450                | Ser Gly Se<br>3460                | r Tyr Arg V<br>3470        |                    |
| Leu Asp Tyr<br>34                 | C TGG GCC C<br>r Trp Ala A<br>490 | * *<br>GA CCA GGC (<br>rg Pro Gly F<br>3500 | Pro Phe Ser<br>3510               | GAC CCT GT<br>Asp Pro Va<br>3520  | l Pro Tyr L<br>3530        | eu Glu Val<br>3540 |
|                                   |                                   | CC CCA TCC (<br>ro Pro Ser F                |                                   |                                   | * *<br>CTGTGCTGA G<br>3600 |                    |
| * * GTŢGCACCTC 3620               | CACCGGCAGT                        | CAGCGAGCTG<br>3640                          | GGGCTGCACT 3650                   | GTGCCCATGC<br>3660                | TGCCCTCCCA                 |                    |
| TGCAATATAT<br>3690                |                                   | TAAAAAAAAA<br>3710                          |                                   |                                   |                            |                    |
| 3760<br>* *                       | 3770<br>* *                       |   | 3790<br>* *                       | 3800<br>* *                       | 3810<br>* *                | 3820<br>* *        |
| 383 <b>0</b><br>* *               | 3840<br>* *                       | 3850  | 3860<br>* *                       | 3870                              | 3880                       | 389 <b>0</b>       |
|                                   |                                   | * * GGGGTGGGGT                              |                                   | 3940<br>* *<br>AGCAAGGGGG<br>4010 |                            | * * AGACAATAGC     |
| 3970<br>* *<br>AGGCATGCTG<br>4040 |                                   | * * GGGCTCTATG                              | 4000<br>* *<br>GCTTCTGAGG<br>4070 | * * CGGAAAGAAC                    | * *                        | * * TCGAGAGCTT     |
| GGCGTAATCA 4110                   | * * TGGTCATAGC 4120               | TGTTTCCTGT<br>4130                          | GTGAAATTGT<br>4140                | TATCCGCTCA 4150                   |                            |                    |
|                                   | TAAAGTGTAA                        | AGCCTGGGGT<br>4200                          | GCCTAATGAG                        | TGAGCTAACT<br>4220                | CACATTAATT<br>4230         | GCGTTGCGCT<br>4240 |
| 4250<br>* *                       | 4260<br>* *                       |   | 4280<br>* *                       | 4290<br>* *                       | 4300<br>* *                | 4310<br>* *        |
| 4320<br>* *                       | 4330<br>* *                       | 4340  | 4350<br>* *                       | 4360                              | 4370<br>* *                | 4380<br>* *        |

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| 4390            | 4400          | 4410<br>* *        | 4420<br>* *        | 4430<br>* *     | 4440<br>* *        | 4450<br>* *        |
|-----------------|---------------|--------------------|--------------------|-----------------|--------------------|--------------------|
|                 |               |                    |                    |                 | GCGTTGCTGG         |                    |
| 4460            | 4470<br>* *   | 4480               | 4490               | 4500            | 4510               | 4520               |
| 4530            | 4540          | 4550               | 4560               | 4570            | GGTGGCGAAA<br>4580 | 4590               |
| * *             | * *           | * *                | * *                | * *             | * * TGTTCCGACC     | * *                |
| 4600            | 4610          | TCCCCCTGGA<br>4620 | 4630               | 4640            | 4650               | 4660               |
| * *             | * *           | * *                | * *                | * *             | * *                | * *                |
| 4670            | 4680          | 4690               | 4700               | 4710            | TGCTCACGCT 4720    | 4730               |
| * *             | * *           | * *                | * *                | * *             | * *                | * *                |
| CAGTTCGGTG      | TAGGTCGTTC    | GCTCCAAGCT         | GGGCTGTGTG         | CACGAACCCC      | CCGTTCAGCC         | CGACCGCTGC         |
| 4740            | 4750          | 4760<br>* *        | 4770<br>* *        | 4780·           | 4790<br>* *        | 4800<br>* *        |
|                 |               |                    |                    |                 | ATCGCCACTG         |                    |
| 4810            | 4820          | 4830               | 4840               | 4850            | 4860               | 4870               |
| * *             | * *           | * *                | * *                | * *             | * *                | * *                |
|                 |               |                    |                    | TACAGAGTTC 4920 | TTGAAGTGGT<br>4930 | GGCCTAACTA<br>4940 |
| 4880<br>* *     | * 4890<br>* * | 4900<br>* *        | 4910<br>* *        | * *             | * *                | * *                |
| CGGCTACACT      | AGAAGGACAG    | TATTTGGTAT         | CTGCGCTCTG         | CTGAAGCCAG      | TTACCTTCGG         | AAAAAGAGTT         |
| 4950            | 4960          | 4970               | 4980               | 4990            | 5000               | 5010               |
| * *             | * *           | * *                | * *                | * *             | * *                | * *                |
|                 |               | ACAAACCACC<br>5040 | GCTGGTAGCG<br>5050 | 5060            | TGTTTGCAAG<br>5070 | 5080               |
| 5020<br>* *     | 5030<br>* *   | * *                | * *                | * *             | * *                | * *                |
| CGCGCAGAAA      | AAAAGGATCT    | CAAGAAGATC         | CTTTGATCTT         | TTCTACGGGG      | TCTGACGCTC         | AGTGGAACGA         |
| 5090            | 5100          | 5110               | 5120               | 5130            | 5140               | 5150               |
| * *             | * *           | * *                | * *                | * *             | * *                | * *                |
| AAACTCACGT 5160 | 5170          | 5180               | 5190               | 5200            | CCTAGATCCT<br>5210 | 5220               |
| * *             | * *           | * *                | * *                | * *             | * *                | * *                |
| AAATGAAGTT      | TTAAATCAAT    | CTAAAGTATA         | TATGAGTAAA         | CTTGGTCTGA      | CAGTTACCAA         | TGCTTAATCA         |
| 5230            | 5240          | 5250               | 5260               | 5270            | 5280               | 5290               |
| * *             | * *           | * *                | * *                | * *             | * *                | * *<br>TCCTCTACAT  |
| 5300            | 5310          |                    |                    |                 | TGACTCCCCG<br>5350 | 5360               |
| * *             |               |                    |                    |                 |                    |                    |
| AACTACGATA      | CGGGAGGGCT    | TACCATCTGG         | CCCCAGTGCT         | GCAATGATAC      | CGCGAGACCC         | ACGCTCACCG         |
| 5370            | 5380          | 5390               | 5400               |                 | - <del>-</del> -   |                    |
| * *             | * *           |                    |                    |                 | * *<br>*           |                    |
| 5440            | 5450          |                    | 5470               |                 | AAGTGGTCCT<br>5490 |                    |
| * *             | * *           | * *                |                    |                 |                    | * *                |
| CCGCCTCCAT      | CCAGTCTATT    | AATTGTTGCC         | GGGAAGCTAG         | AGTAAGTAGT      | TCGCCAGTTA         | ATAGTTTGCG         |

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| 5510       | 5520       | 5530       | 5540       | 5550       | 5560       | 5570       |
|------------|------------|------------|------------|------------|------------|------------|
| * *        | * *        | * *        | * *        | * *        | * *        | * *        |
| CAACGTTGTT | GCCATTGCTA | CAGGCATCGT | GGTGTCACGC | TCGTCGTTTG | GTATGGCTTC | ATTCAGCTCC |
| 5580       | 5590       | 5600       | 5610       | 5620       | 5630       | 5640       |
| * *        | * *.       | * *        | * *        | * *        | * *        | * *        |
| GGTTCCCAAC | GATCAAGGCG | AGTTACATGA | TCCCCCATGT | TGTGCAAAAA | AGCGGTTAGC | TCCTTCGGTC |
| 5650       | 5660       | 5670       | 5680       | 5690       | 5700       | 5710       |
| * *        | * *        | * *        | * *        | * *        | * *        | * *        |
| CTCCGATCGT | TGTCAGAAGT | AAGTTGGCCG | CAGTGTTATC | ACTCATGGTT | ATGGCAGCAC | TGCATAATTC |
| 5720       | 5730       | 5740       | 5750       | 5760       | 5770       | 5780       |
| * *        | * *        | * *        | * *        | * *        | * *        | * *        |
| TCTTACTGTC | ATGCCATCCG | TAAGATGCTT | TTCTGTGACT | GGTGAGTACT | CAACCAAGTC | ATTCTGAGAA |
| 5790       | 5800       | 5810       | 5820       | 5830       | 5840       | 5850       |
| * *        | * *        | * *        | * *        | * *        | * *        | * *        |
| TAGTGTATGC | GGCGACCGAG | TIGCTCTTGC | CCGGCGTCAA | TACGGGATAA | TACCGCGCCA | CATAGCAGAA |
| 5860       | 5870       | 5880       | 5890       | 5900       | 5910       | 5920       |
| ** *       | * *        | * *        | * *        | * *        | * *        | . * *      |
| CTTTAAAAGT | GCTCATCATT | GGAAAACGTT | CTTCGGGGCG | AAAACTCTCA | AGGATCTTAC | CGCTGTTGAG |
| 5930       | 5940       | 5950       | 5960       | 5970       | 5980       | 5990       |
| * *        | * *        | * *        | * *        | * *        | * *        | * *        |
| ATCCAGTTCG | ATGTAACCCA | CTCGTGCACC | CAACTGATCT | TCAGCATCTT | TTACTTTCAC | CAGCGTTTCT |
| 6000       | 6010       | 6020       | 6030       | 6040       | 6050       | 6060       |
| * *        | * *        | * *        | , * *      | * *        | * *        | * *        |
| GGGTGAGCAA | AAACAGGAAG | GCAAAATGCC | GCAAAAAAGG | GAATAAGGGC | GACACGGAAA | TGTTGAATAC |
| 6070       | 6080       | 6090       | 6100       | 6110       | 6120       | 6130       |
| * *        | * *        | * *        | * *        | * *        | * *        | * *        |
| TCATACTCTT | CCTTTTTCAA | TATTATTGAA | GCATTTATCA | GGGTTATTGT | CTCATGAGCG | GATACATATT |
| 6140       | 6150       | 6160       | 6170       | 6180       | 6190       | 6200       |
| * *        | * *        | * *        | * *        | * *        | * *        | * *        |
| TGAATGTATT | TAGAAAAATA | AACAAATAGG | GGTTCCGCGC | ACATTTCCCC | GAAAAGTGCC | ACCTGACGTC |

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Title: Methods of Producing and Purifying Recombinant Alpha-L-Iduronidase

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Fig. 2A

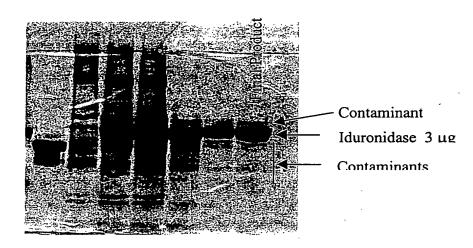
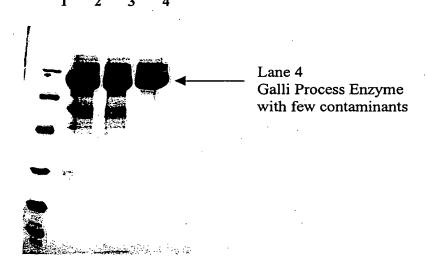


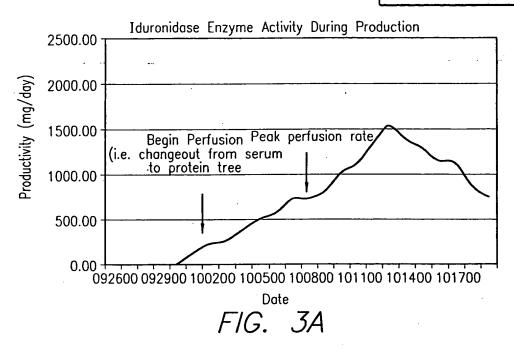
Fig. 2B

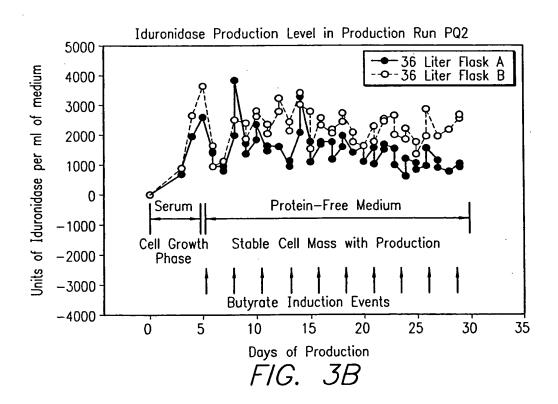


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Iduronidase
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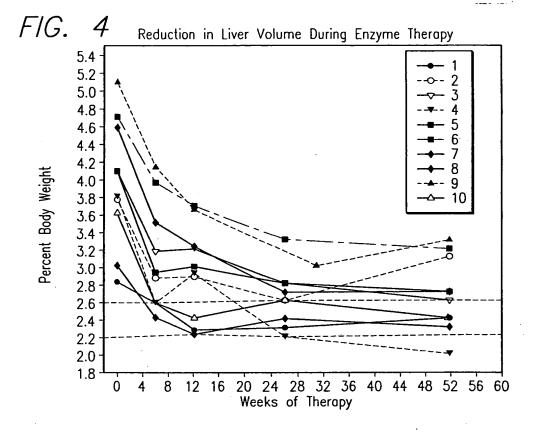
— Productivity (mg/day)

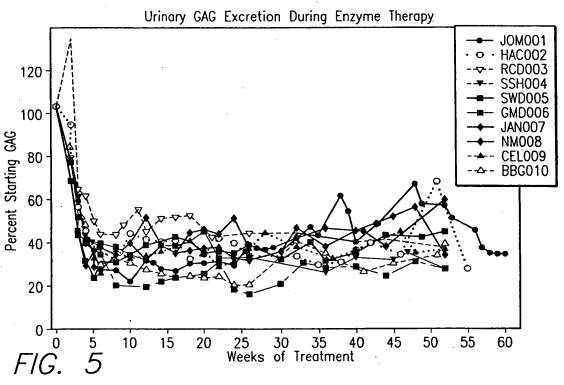




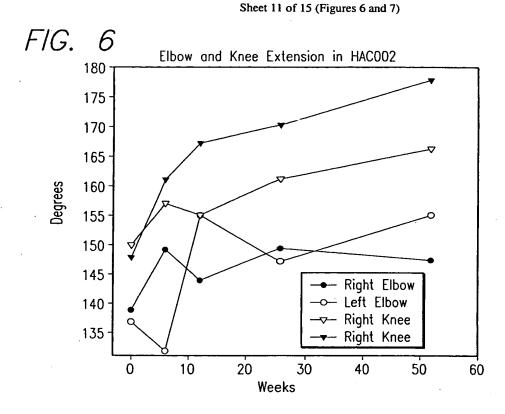
U.S. Application No. 10/722,371 Inventors: Qin et al. Title: Methods of Producing and Purifying Recombinant Alpha-L-Iduronidase Docket No. 30610/30009A

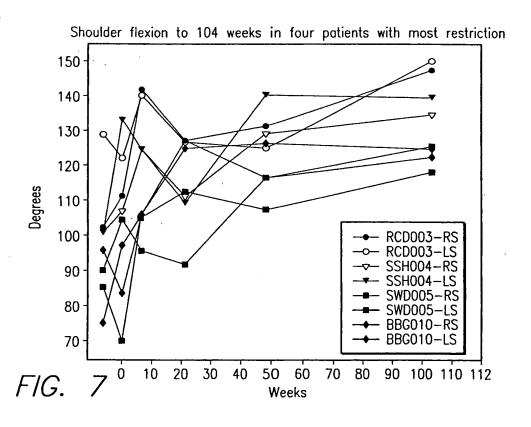
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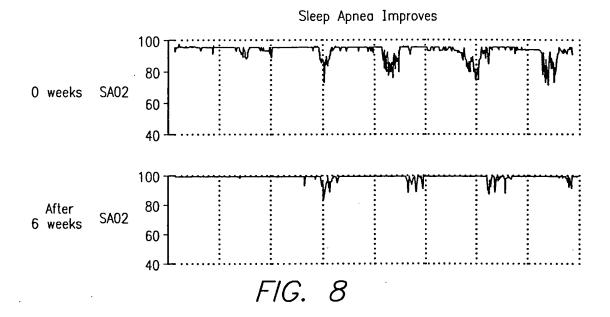


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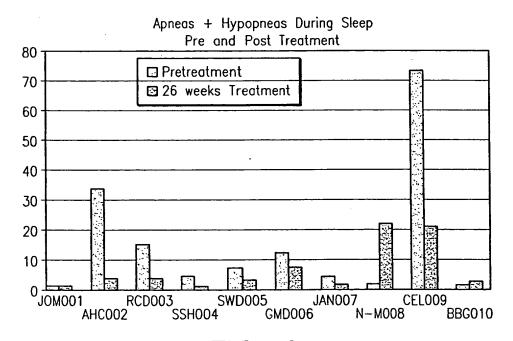
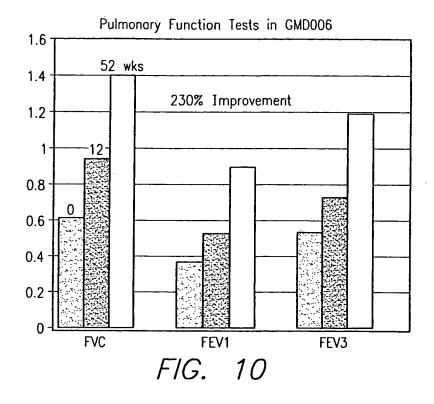
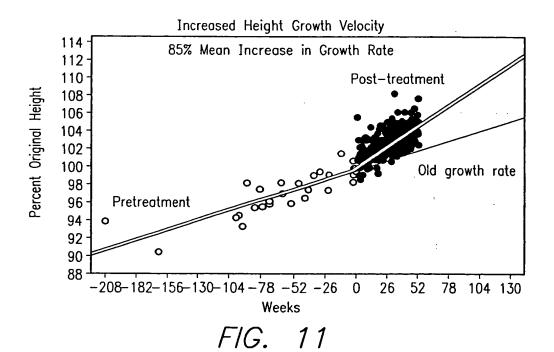


FIG. 9

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F/G. 12
Chinese Hamster Ovary Host Protein Contamination by ELISA Assay

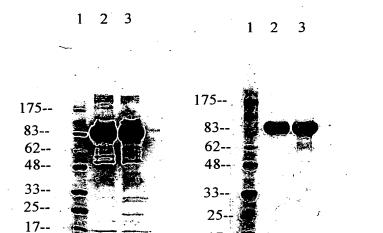
| SOURCE AND BATCH<br>NUMBER | CHOP PROTEIN CONTAMINATION (microgram per milligram) | PERCENT CHOP CONTAMINATION | PURITY OF THE<br>ENZYME FROM<br>CHOP |
|----------------------------|--|----------------------------|--------------------------------------|
| Prior Process (Carson/REI) |  |                            |                                      |
| C9002                      | 14   | 1.4%                       | 98.6%                                |
| C9003                      | 24   | 2.4%                       | 97.6%                                |
| C9004                      | 16   | 1.6%                       | 98.4%                                |
| New Process (Galli)        |  | 1                          |                                      |
| P1003                      | <1.3   | <0.13%                     | >99.9%                               |
| P1006                      | 1.2  | 0.12%                      | 99.9%                                |
| P1007                      | <0.6   | <0.06%                     | >99.9%                               |
| P1008                      | <0.67  | <0.067%                    | >99.9%                               |

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### Comparison of Galli and Carson Material



anit-IDU Western blot 1:50,000

SDS-PAGE silver stain

- 1 Marker
- 2 Galli Referenced-0201
- 3 Carson C9002

5ug/lane

FIG. 13